IN THE MATTER OF U.S. Patent Application No.: 10/537,002 assigned to Ganymed Pharmaceuticals AG

DECLARATION OF PROF. DR. UGUR SAHIN
UNDER 35 C.F.R. § 1.132

I, **UGUR SAHIN**, Chief Medical Officer of **GANYMED Pharmaceuticals AG, Freiligrathstrasse 12, D-55131 Mainz, Germany,** and Professor for Experimental Oncology, University of Mainz, hereby declare that:

1 I am one of the inventors named in the above-identified patent application, and I am familiar with its contents.

2 I obtained my Medical Doctor in Medicine at the University of Cologne in 1990. After a period as Junior Resident at the Dpt. of Medicine I changed to the University Hospital Homburg/Saar, Dpt. of Internal Medicine, as a Senior Resident. In 1993 I became a Research group head and have invented the SEREX technology, which is a molecular biological method for identifying and analysing tumor antigens. In 1995 I was awarded the Vincent Czerny Cancer Research Award of the German Association of Hemato-Oncology for the development of the SEREX technology, and in 2005 I was awarded the George Köhler Prize of the German Association for Immunology (DGfI) for the development of innovative concepts for antigen-specific cancer immunotherapy. Since 2001 I was heading Research Groups at the University Hospital Mainz, working in the field of identification, characterization and validation of tumor antigens among other things, using molecular biological, cellular and biochemical methods. In 2001 I have co-founded Ganymed Pharmaceuticals AG and became chairman of the Scientific Advisory Board, and since 2007 I am Chief Medical Officer working on preclinical and clinical development of antibodies specific for tumor antigens. Since 2006 I am Professor for Experimental Oncology, University Hospital Mainz, attending in the Dpt. of Internal Medicine III.

3 I have read and am familiar with the Office Actions issued by the U.S. Patent and Trademark Office ("USPTO") with respect to the above patent application. I make this Declaration in support of the patentability of the instant invention. The purpose of this Declaration is to make certain scientific observations on mRNA-protein correlation.

4 The above patent application is directed to a method of diagnosing cancer, comprising detecting expression of a tumor-associated antigen in a biological sample, wherein the tumor-associated antigen is selected from the group consisting of (i) the polypeptide SEQ ID NO: 16 (Claudin18A2.1) and (ii) the polypeptide encoded by the nucleic acid sequence SEQ ID NO: 7, wherein detection of the tumor-associated antigen in a biological sample isolated from a patient in an amount greater than an amount of the tumor-associated antigen in a normal biological sample indicates the presence of cancer.

In the above patent application, it is shown, based on RT-PCR experiments, that Claudin18A2.1 is, for example, not expressed in normal pancreas, lung, or esophagus tissue, however, expression was observed for the respective tumor tissues, i.e., pancreas carcinoma, bronchial carcinoma (lung carcinoma), and esophagus carcinoma tissues, respectively; cf. Table 3A of the above patent application.

Furthermore, it is shown that the existence of Claudin18A2.1 mRNA in lung carcinoma tissue also reflects the presence of Claudin18A2.1 protein in said tissue; cf. Figures 24 (mRNA in normal and cancerous tissue), 29 (protein in normal tissue), and 31 (protein in cancerous tissue). Thus, these data show that in the case of Claudin18A2.1, the presence of mRNA is indicative of the presence of corresponding protein.

5 The Examiner alleges that protein expression cannot be predicted based on RNA expression data and cites for support of this statement Greenbaum et al. (2003, Genome Biology 4:117.1-117.8), Brennan et al. (1989, J. Autoimmunity 2 (suppl.): 177-186), Zimmer (1991, Cell Motility and the Cytoskeleton 20:325-337), Hell et al. (1995, Laboratory Investigation 73:492-496), Fu et al. (1996, EMBO J. 15:4392-

4401), Vallejo et al. (2000, Biochimie 82:1129-1133), and Jang et al. (1997, Clinical Exp. Metastasis 15:469-483); cf. pages 12 to 14 of the Office Action dated October 17, 2006; page 4, 2nd paragraph of the Office Action dated June 6, 2007; page 4, 2nd paragraph of the Office Action dated February 14, 2008.

I respectfully disagree with the Examiner's contentions for the reasons set forth below.

5.1 Greenbaum et al. studied correlations between mRNA and protein abundance for a set of protein abundance information of approximately 2,000 ORFs; cf. page 117.4, right column, lines 6 to 8 and 47 to 51. In other words, Greenbaum et al. investigated the correlation between the relative amounts of mRNA and the relative amounts of protein such that a perfect positive correlation (i.e., r = 1) would mean: mRNA A is 3-fold as abundant as mRNA B and protein A is 3-fold as abundant as protein B. In this context, Greenbaum et al. found that there is a high degree of positive mRNA-protein abundance correlation (r = 0.89) for ORFs that show a large degree of variation in their expression during the cell cycle, while those ORFs that show minimal variation in their mRNA expression during the cell cycle show a minimal correlation of r = 0.2; cf. page 117.4, right column, line 47 to page 117.5, right column, line 11.

In this context, it should be noted that even for ORFs that show less correlation, the correlation is positive. This indicates that qualitatively an increased amount of mRNA also represents an increased amount of protein, even if the data do not correlate quantitatively.

While Greenbaum et al studied a quantitative correlation of mRNA with protein amounts, the instant patent application teaches a positive correlation between the presence of mRNA with the presence of the corresponding protein, that is, the presence of mRNA indicates the presence of protein, while the absence of mRNA indicates the absence of protein. Thus, in the instant application, it is only absence or presence of mRNA that should be indicative of the absence or presence of protein without regard to any quantitative correlation.

Furthermore, as mentioned previously, it has been shown for Claudin18A2.1 in the above patent application that presence of mRNA is indicative for presence of Claudin18A2.1 protein based on the example of lung cancer.

5.2 Brennan et al. have observed that while lymphotoxin (LT) and interferon γ (IFNγ) mRNA levels are high in synovial cell cultures isolated from diseased joints of patients with rheumatoid arthritis, the respective proteins were not detected. Brennan et al. note with respect to this phenomenon that "more sensitive assays are clearly needed to explore this further"; cf. page 182, paragraph under the heading "LT and IFNγ gene expression". Therefore and since this publication is almost 20 years old, it appears that the apparent absence of protein is rather due to the insensitivity of the assay than due to an actual absence of protein.

5.3 Zimmer reports on a study investigating the level, subcellular distribution, and potential target proteins of the S100 family of calcium-modulated proteins in adult and developing rat skeletal muscle. Zimmer observes that while the distribution of S100 mRNAs paralleled the protein distribution in all muscles, there was no direct correlation between the mRNA and protein levels in different muscle types; cf. abstract. Thus, Zimmer clearly observes that the presence of mRNA is indicative for the presence of protein (qualitatively); cf. section 3.1, hereinabove.

5.4 Hell et al. describe a study on the expression of the bcl-2 oncogene in Hodgkin's disease both at the protein and mRNA level in correlation with the expression of the Eppstein-Barr virus-encoded late membrane protein. The main finding was that the vast majority of Hodgkin's cells, irrespective of subtype, express abundant bcl-2 mRNA, while oncogene expression varied from case to case; cf. abstract.

However, it appears that – while protein levels may vary – the presence of mRNA is indicative for the presence of protein in the study described by Hell et al.

5.5 Fu et al. report on a study on translational regulation of human p53 gene expression and find that while p53 mRNA was present in all samples examined, the expression of

p53 protein was variable from patient to patient and that the level of p53 mRNA and the level of p53 protein expression in blast cells did not correlate; cf., e.g., abstract.

P53 is a transcription factor and thus belongs to the category of regulatory proteins. Guo et al. (2008, Acta Biochim Biophys Sin 40:426-436), however, have shown that genes belonging to the category of regulation had non-significant and lowest mRNA-protein expression correlations; cf., e.g., abstract of Guo et al.

In the case of the above patent application, Claudin18A2.1 is not a regulatory but rather a structural protein. Thus, the phenomenon observed for p53 cannot be applied to Claudin18A2.1, in particular, since it has been shown in the above patent application that presence of Claudin18A2.1 mRNA is indicative for presence of Claudin18A2.1 protein.

5.6 Vallejo et al. have analyzed the expression of NRF-2 subunits both at the mRNA and at the protein level in three rat tissues, liver, testis, and brain, and they have found that there was no correlation between NRF-2 mRNA and protein levels; cf. abstract. However, they clearly show that all samples do express NRF-2 protein, and thus, also in this case and although NRF-2 is a regulatory protein (cf. section 3.5, hereinabove) the presence of mRNA is indicative for the presence protein; cf. Figure 1 and Table I. Furthermore, Vallejo et al. also show that there is perfect correlation between the mRNA and protein levels for Tfam and β-actin; cf. Figure 2 and Table I.

5.7 Jang et al. investigated the effects of hypoxia, acidosis, and glucose starvation on the expression of metastasis-associated genes in murine tumor cells. However, it appears that Jang et al. have not studied the correlation of mRNA and protein levels, since they state that further studies are required to establish whether changes in protein levels track with changes in mRNA levels for the investigated genes; cf. abstract. Thus, Jang et al. cannot serve to substantiate the Examiner's argument.

6 Furthermore, there appears to be a consensus opinion in the field that even though the amount of mRNA may not reflect the amount of protein present in a cell, presence of mRNA is generally also indicative for presence of protein.

6.1 Tian et al. (2004, Molecular & Cellular Proteomics 3:960-969) report on integrated genomic and proteomic analyses of gene expression in mammalian cells and observe that the overall pattern of protein expression is similar to that of mRNA expression and that the correlation coefficient is clearly positive, i.e., 0.59 for all genes examined; cf. abstract and page 962, left column, line 40 to right column, line 8.

6.2 Shankavaram et al. (2007, Mol. Cancer Ther. 6:820-832) describe an integromic microarray study on transcript and protein expression profiles of the NCI-60 cancer panel and have shown that there are two groups of proteins with different mRNA-protein correlation coefficients, i.e., group 1 with a mean r = 0.71 and group 2 with a mean r = 0.28. In this respect, Shankavaram et al. report that genes in the structural category were most significantly associated with high correlations, whereas genes in the signal transduction category were significantly associated with low, but still positive, correlations; page 828, right column, lines 34 to 37 and 50 to 55.

Since Claudin18A2.1 is rather grouped into the structural category than the signal transduction category, Claudin18A2.1 should – according to Shankavaram et al. – not only exhibit a qualitative correlation but also a quantitative correlation between mRNA and protein levels.

Furthermore, Shankavaram et al. state: "Because the transcript-based technologies are more mature and are currently able to assess larger numbers of genes at one time, they continue to be useful, even when the ultimate aim is information about proteins"; cf. abstract. Thus, Shankavaram et al. confirm that, in general, it is scientifically permissible to extrapolate from information on the RNA status to the protein status.

6.3 Orntoft et al. (2002, Molecular & Cellular Proteomics 1:37-45) report on a genome-wide study of gene copy numbers, transcripts, and protein levels in pairs of non-

invasive and invasive human transitional cell carcinomas and found that there was in general a highly significant correlation between transcript alterations and protein levels; cf. abstract, Figure 4, page 42, right column, lines 4 to 6 of the main text.

6.4 Guo et al. (2008, Acta Biochim. Biophys. Sin. 40:426-436) describe a study on how mRNA expression is predictive for protein expression based on data obtained from human circulating monocytes. Their results showed an overall positive correlation between mRNA and protein expression levels. The highest correlation was observed for genes of the extracellular region in terms of cellular components and the lowest correlation was observed for genes of regulation in terms of biological processes; cf. abstract, page 429, right column, line 49 to page 432, left column, line 6, and page 434, left column, line 39 to right column, line 5. Guo et al. conclude from their data that the number of mRNA copies may not reflect the number of functional protein molecules, however, mRNA expression is still informative to protein expression; cf. page 435, right column, lines 31 to 46.

Summarizing the above, there is a general consensus in the field that even if the amount of mRNA does not always exactly reflect the amount of protein encoded by said mRNA, the presence of mRNA can be considered indicative for the presence of protein. Furthermore, genes of structural proteins, such as Claudin18A2.1, are rather transcriptionally than translationally regulated, and thus, higher mRNA-protein correlation coefficients are generally observed for such proteins.

In view of the fact that (i) Claudin18A2.1 mRNA is not present at all in normal tissues except testis and stomach but is present in considerable amounts in several cancer types, (ii) there is no indication for a missing correlation between Claudin18A2.1 mRNA and protein expression and (iii) a positive correlation between Claudin18A2.1 mRNA and protein expression for normal lung tissue and lung cancer tissue, respectively, has been demonstrated in the above patent application, it is evident that Claudin18A2.1 protein is a useful marker in the diagnosis of cancer for which presence of Claudin18A2.1 mRNA is characteristic.

7 I declare under penalty of perjury that the foregoing is true and correct.

Signed .....

Prof. Dr. Ugur Sahin

July 3rd, 2008